Review

Mechanisms of long COVID and the path toward therapeutics

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SUMMARY

Long COVID, a type of post-acute sequelae of severe acute respiratory syndrome coronavirus 2 SARS-CoV-2) PASC) defined by medically unexplained symptoms following infection with SARS-CoV-2, is a newly recognized infection-associated chronic condition that causes disability in some people. Substantial progress has been made in defining its epidemiology, biology, and pathophysiology. However, there is no cure for the tens of millions of people believed to be experiencing long COVID, and industry engagement in developing therapeutics has been limited. Here, we review the current state of knowledge regarding the biology and pathophysiology of long COVID, focusing on how the proposed mechanisms explain the physiology of the syndrome and how they provide a rationale for the implementation of a broad experimental medicine and clinical trials agenda. Progress toward preventing and curing long COVID and other infection-associated chronic conditions will require deep and sustained investment by funders and industry.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection can affect long-term health. Although COVID-19 in most individuals is self-limited, a proportion of those infected experience post-acute sequelae, including (1) the post-hospital or post-intensive care unit (ICU) syndrome characterized by muscle weakness, fatigue, and cognitive dysfunction; (2) newly developed or exacerbated medical conditions such as diabetes mellitus, cerebrovascular events, and autoimmune disease; and/ or (3) the emergence of medically unexplained symptoms that affect every day functioning and quality of life.[1,2] While more common following severe COVID-19, the highest burden is among those with a history of mild-to-moderate illness, who comprise the vast majority of infected individuals.[3] These outcomes represent a newly identified infection-associated chronic condition (IACC): long COVID.

DEFINING LONG COVID

There are complex and competing terms and definitions for post-acute COVID-19-related complications. The World Health Organization (WHO) defines "post-COVID-19 condition" as unexplained symptoms lasting at least 2 months and present at least 3 months following SARS-CoV-2 infection.[4] Although many have adopted or adapted the WHO definition, various governmental and public health organizations around the world have also formulated their own, often country-specific, definitions. In the United States (US), the Centers for Disease Control and Prevention (CDC) uses the term "post-COVID-19 conditions,"[5] and the National Institutes of Health uses the term "post-acute sequelae of SARS-CoV-2 (PASC)."[6] In the U.K.,

the National Institute for Clinical Excellence (NICE) prefers "post-COVID-19 syndrome."[7] The Spanish Ministry of Health commissioned a consensus study referring to "post-COVID-19" as "a set of multi-organic symptoms that persist or fluctuate after acute COVID-19 infection and are not attributable to other causes," lasting at minimum 3 months.[8] Similar exercises have been conducted in many other settings, including Germany,[9] Italy,[10] Australia,[11] and South Africa.[12] Efforts to define long COVID in low-income settings have been slower to start,[13] but are also expected to accelerate.

The patient community, who first identified the condition,[14,15] favors the term long COVID, sometimes in reference to unexplained symptoms and sometimes as a synonym for the above terms. Drawing from the expertise of clinicians and those with lived experience, a committee convened by the US National Academies of Sciences, Engineering, and Medicine (NASEM) in 2023–2024 formulated a comprehensive working definition of long COVID.[16] In their report, long COVID is defined as a "chronic disease state" that may be clinically diagnosed after 3 months of symptoms or diagnosable conditions that affect one or more organ systems. Symptoms may vary in severity and can be continuous, relapsing and remitting, or progressive. The authors of this report note the economic or social functional impairment often associated with long COVID, as well as the emotional and physical distress it brings for patients, families, and caregivers.

It is easy to get lost in the nuances of these definitions. Overall, they vary in two key areas: (1) the time scale over which the condition is defined (e.g., 1 versus 3 months post-COVID-19) and (2) whether the condition is inclusive of incident medical diagnoses or laboratory abnormalities (e.g., myocardial infarction [MI] and renal insufficiency) or restricted to unexplained patient-reported
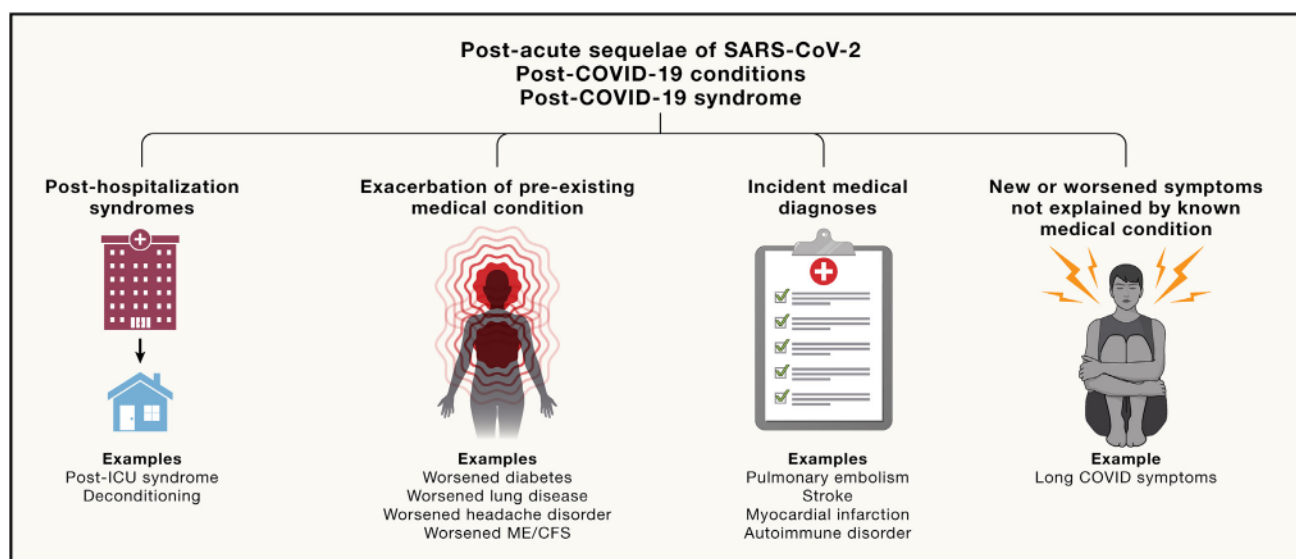
Figure 1   Proposed framework for defining PASC
Until the biology is better understood, we favor a framework using post-acute sequelae of SARS-CoV-2 infection (PASC), as an umbrella term comprising post-hospitalization syndromes, new or worsened medical conditions, and unexplained symptoms (long COVID). We use long COVID to refer specifically to unexplained symptoms. While some biological mechanisms may turn out to overlap, such a framework ensures clarity of case definitions in research studies, limits heterogeneity and misclassification, and maximizes the chances of defining the underlying pathophysiology.

symptoms. As clinicians, we favor a framework that uses an overarching term (e.g., PASC or post-COVID-19 condition) to represent all of the potential complications of SARS-CoV-2 and "long COVID" as a term to represent those symptoms not explained by an alternative diagnosis (Figure 1).

While a broad, inclusive definition for long COVID is needed to ensure access to clinical care, research studies will need to focus more narrowly to avoid heterogeneity, misclassification, and failure to identify a signal (e.g., distinguishing post-COVID-19 MI from otherwise unexplained post-COVID-19 fatigue). Indeed, one challenge in interpreting the long COVID literature to date is that the studies reviewed herein apply a variety of different case definitions and might therefore not be directly comparable to one another.

Research into long COVID has lagged behind the study of acute infection for several reasons. There was delayed recognition by the medical establishment of long COVID as a clinically meaningful entity.[14] Many remain skeptical that the syndrome is real. Long COVID is highly heterogeneous, and each study seemingly uses its own definition. There are no accepted biomarkers or diagnostic tests yet. Given this uncertainty, the biotechnology and pharmaceutical industry has largely remained on the sidelines, citing the lack of an interpretable outcome. Finally, investment in studying long COVID was initially limited, but this has been remedied in part by programs like the NIH's Researching COVID-19 to Enhance Recovery (RECOVER) program, an ambitious $1.4 billion program to study the epidemiology, biology, and treatment of long COVID. The U.K., European Union, Australia, and, to a lesser degree, other countries have also made sizable investments, although there have been many calls for more funding in multiple settings.

Despite the need for sustained momentum,[17] current research programs are starting to pay off, with high-quality mechanistic studies emerging. Rationally selected therapeutic interventions are being advanced through the early clinical development process. We are now entering the era of experimental medicine, in which first-in-human and proof-of-concept clinical trials will shape the agenda. In this review, we summarize the epidemiology of long COVID and then focus on how mechanistic studies are influencing the development of biomarkers, functional tests, and clinical interventions. To build upon recent reviews cataloging these mechanisms,[18–21] we attempt to focus on how the mechanisms might explain the physiology and ultimately inform the treatment of long COVID.

LONG COVID EPIDEMIOLOGY

Epidemiologic estimates of long COVID vary across variants, regions, and populations. Some studies suggest a prevalence of 30% or greater but define the syndrome as any new, unexplained symptom, which results in misclassification of individuals who have symptoms attributable to other conditions. Not all symptoms a person experiences following COVID-19 are causally related to the virus; other conditions must be carefully ruled out (e.g., heart failure, cancer, and thyroid disease). In addition, a significant challenge in long COVID research is determining what is truly new following infection versus what may be considered "unmasking" of a pre-clinical or sub-clinical medical condition. While both are clinically significant, the former is what really represents the condition of interest—an infection-associated syndrome that is fundamentally caused by and specific to COVID-19.
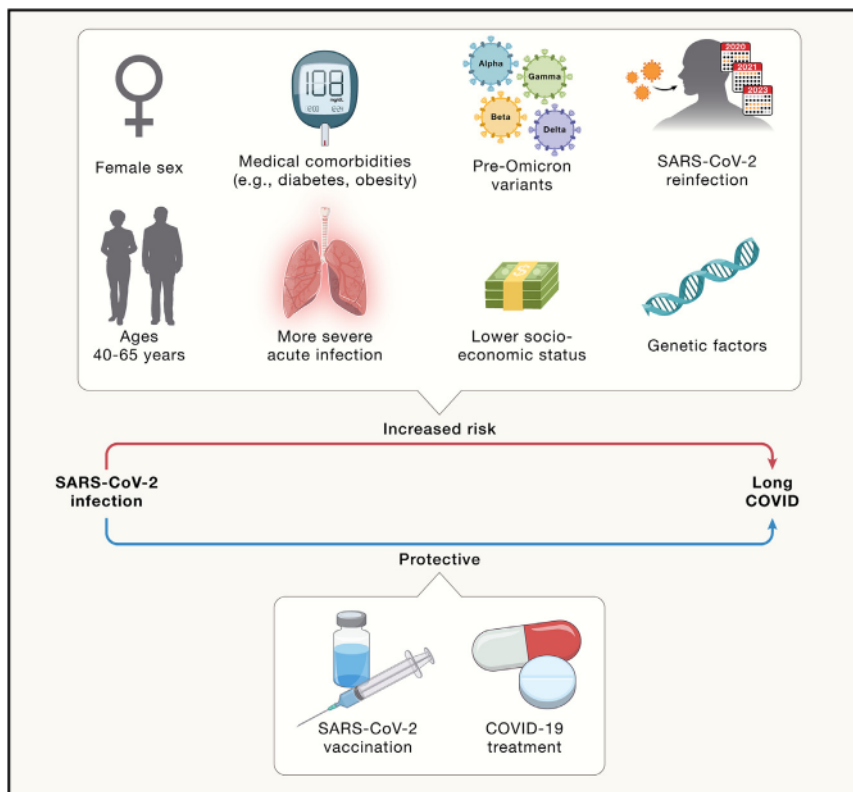
Figure 2 Risk factors and protective factors for long COVID
Several factors have been demonstrated to increase the risk of a person infected with SARS-CoV-2 developing long COVID. More recently, data have emerged supporting the role of SARS-CoV-2 vaccination and treatment in mitigating long COVID.

long COVID between 5% and 10% are emerging in China.[30] Overall, it has been estimated that 65 million people worldwide have long COVID.[15] Even if the incidence is low, this number will likely grow as SARS-CoV-2 continues to spread and as recovery is slow, incomplete, or elusive for many.[31,32]

The epidemiology of the more disabling forms of the condition is largely unknown. Severe, disabling long COVID is uncommon, affecting only a few percent of those infected. But even if it is relatively rare among those exposed to SARS-CoV-2, the syndrome is profoundly important, given the impact it can have on health and the fact that nearly everyone might be expected to be exposed to the virus. Conservative estimates suggest that there may be over 4 million people with profound disease in the US alone.[33] Even a small risk of long COVID represents a major public health concern.

The risk factors for developing long COVID have been consistent: female sex, older age, more severe acute infection, and lower socioeconomic status (Figure 2).[34–37] Genetic and epigenetic factors may also play a role.[38–41] Although the highest incidence was associated with the first waves of the pandemic, long COVID has continued with the emergence of novel variants and in spite of the widespread implementation of vaccines and rollout of antiviral therapy. The degree to which SARS-CoV-2 variants differ in their capacity to cause long COVID is unknown, but some studies suggest the risk after Omicron is low relative to Delta and earlier variants.[42] The effect of reinfection is unclear, but at least one study suggests harm.[43] Vaccination is protective but does not fully prevent long COVID[44–47]; further work is needed to determine the additive benefit of booster doses in the era of modern variants. Early antiviral treatment appears beneficial in some populations,[48,49] although the data are mixed in others.[50,51] Despite our growing epidemiologic understanding of risk as well as evidence that the early viral burden may predict long-term outcomes,[52–54] the long COVID prevention agenda remains limited.

CLINICAL FEATURES OF LONG COVID

Long COVID symptoms can emerge during the acute phase of infection or later, after several weeks. The most common symptoms include fatigue, cognitive problems, and post-exertional symptoms.[15] Symptoms can wax and wane.[55–57] To date,

SARS-CoV-2 is also not the only virus associated with prolonged symptoms. Indeed, when a control uninfected population is included, the prevalence of new symptoms is often about half of that in the post-COVID-19 population[22]; these symptoms might be due to other infections (e.g., influenza, etc.) or non-infectious issues.[23] However, in comparison to other viruses, COVID-19 appears to exert a disproportionate effect on long-term health. For example, a study in the US Veterans' Affairs health system that compared long-term outcomes following COVID-19 versus influenza found higher risks of adverse outcomes across multiple organ systems in the post-COVID-19 group.[24] Recent efforts have been made to distinguish symptoms that are more common among those with long COVID in comparison to the general population following other illnesses[22] or against uninfected controls.[6]

While overly inclusive research definitions have fueled skepticism about the scale of the problem,[25] that some studies have overestimated the prevalence of long COVID should not be used to diminish its importance. Rigorous studies have estimated that 18 million US adults might be experiencing long COVID,[26] in line with CDC estimates that 6% are experiencing it currently.[27] Population-based surveys in the UK have carefully tracked long COVID over time; as of March 2023, 2.9% of the population met the case definition for long COVID, including 1.3 million people who had COVID-19 more than a year prior.[28] The Institute for Health Metrics and Evaluation and WHO estimated that 1 in 30 people across Europe experienced long COVID in the first 3 years of the pandemic.[29] Similar data suggesting the prevalence of
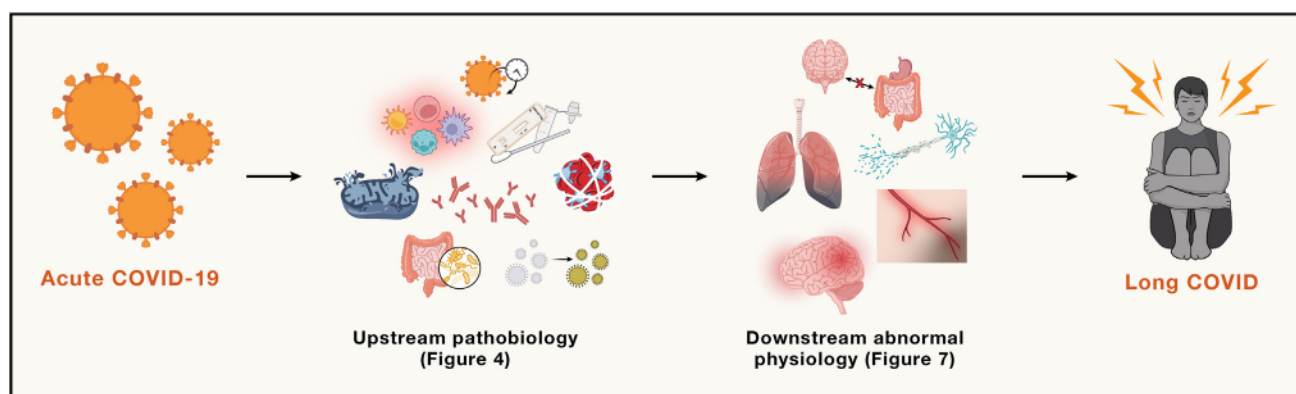
Figure 3 Conceptual model for the mechanisms driving long COVID
Following SARS-CoV-2 infection, abnormal biologic processes related to the interaction between the virus and host interact to drive abnormal physiologic function, which is ultimately experienced as symptoms of long COVID.

most assessments of long COVID have focused on organ system-associated symptoms based upon a clinical assessment. In the RECOVER network of clinical trials, the NIH is focusing on clinical endotypes such as autonomic dysfunction, neurocognitive impairment, and exercise intolerance. Such endotypes are presumably driven by unique but potentially overlapping upstream biological mechanisms that may eventually prove to be the target of interventions.

The field is moving toward more objective associations between symptoms using techniques like cluster analysis; this is an important step as it is possible that disparate symptoms may turn out to be biologically related even in the absence of a clear organ system association. Several groups have characterized subtypes of long COVID using electronic health record (EHR) data. One such analysis identified four endotypes: cardiac/renal, respiratory/sleep/anxiety, musculoskeletal/neurologic, and digestive/respiratory.[58] These analyses are only as good as the quality of the health record (e.g., accurate diagnostic coding and complete review of systems). Other smaller studies have identified similar clusters, or clustering based upon number of symptoms.[55,56] Currently, many endotypes exhibit overlapping features, making it difficult to apply them in practice. Delineating objectively defined phenotypes that can be easily applied in a clinical setting would be helpful; for now, clinical assessment must suffice.

Many long COVID symptoms overlap with those seen in other IACCs such as myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), chronic Lyme disease, post-Giardiasis and post-Ebola disease, among others.[59–64] Historically, these conditions have received limited resources due to the same barriers that prevented early investigation of long COVID, but that seems to be changing. It is hoped that the knowledge gained in the defining the biology and treatment of long COVID will have collateral benefits for other IACCs.

A CONCEPTUAL FRAMEWORK FOR THE MECHANISMS OF LONG COVID

Many mechanisms of long COVID have been proposed. To date, these have largely been studied and discussed in isolation. Individual publications frequently focus on providing evidence for or against a particular biological pathway or physiologic abnormality. However, human biology and physiology are complex, and it is almost certainly the case that these mechanisms are not mutually exclusive but are in fact highly intertwined. Indeed, it will be critical in the next phase of research to determine the relationships between different contributors to long COVID. Here, we propose a conceptual framework that makes a distinction between biology and physiology—which we refer to as the upstream and downstream mechanisms (Figure 3). In doing so, our goal is to explain how SARS-CoV-2 infection can cause long COVID and to identify potential therapeutic targets for each mechanism.

UPSTREAM: BIOLOGICAL DRIVERS OF LONG COVID

The biological drivers of long COVID are upstream processes—perturbations of the immune system, coagulation system, etc.—that in and of themselves do not clearly cause disease (Figure 4). Instead, these processes may drive one another (Figures 5 and 6), as well as downstream physiologic changes (Figure 7) that manifest as symptoms or syndromes in people with long COVID. Below, we discuss multiple biological drivers, each section starting with the proposed pathobiology, followed by a discussion of therapeutic targets.

The consequences of acute infection
SARS-CoV-2 can infect a variety of cell types, including the epithelial cells of the respiratory tract[65] and enterocytes in the gastrointestinal tract.[66] Vascular endothelial cells express ACE2, although whether they can support viral replication remains controversial;[67] still, direct infection of the endothelium or indirect effects from the inflammatory cascade could lead to systemic endotheliitis and many of the systemic symptoms associated with acute disease. Other tissues (e.g., heart, kidneys, and adipose) express ACE2 and may support active replication,[68] but this remains less well-characterized. Whether cells within the brain can be infected is controversial, although fragments of SARS-CoV-2 have been found during autopsy.[69] Residual and
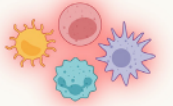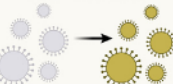
Biological mechanisms of Long COVID

	Research testing	Therapeutics for evaluation
Acute infection (prevention)	COVID-19 testing Viral dynamics	Vaccination COVID-19 antivirals Monoclonals Metformin Anti-inflammatories Anti-platelet drugs Anticoagulants
Virus persistence	Blood antigen Blood PCR Tissue biopsy	Vaccination (therapeutic) COVID-19 antivirals Monoclonals
Post-acute inflammation	Blood markers (e.g., CRP) Cellular immunology Proteomics Radiographic imaging PET imaging Tissue biopsy	JAK/STAT inhibitors Interleukin inhibitors Inflammasome inhibitors Checkpoint inhibitors Rapamycin
Autoimmunity	Blood markers (e.g., ANA) Autoantibody testing B cell testing	IVIG Plasmapheresis B cell depletion
Thrombosis	Blood markers (e.g., fibrinogen, D-dimer) "Microclot" assays	Anti-platelet drugs Anticoagulants Thrombolytics Plasmapheresis
Latent virus reactivation	EBV PCR EBV serology EBV cellular assays VZV assays HHV-6 assays	Antivirals (e.g., ganciclovir) EBV cellular therapies
Dysbiosis and gut translocation	Microbial markers (e.g., B-glucan, LPS, zonulin) Fecal microbiota	Probiotics Larazotide Fecal transplant
Mitochondrial dysfunction	Mitochondrial proteins Reactive oxygen species Muscle biopsy	Amino acids N-acetylcysteine Metformin

Figure 4   Biological mechanisms of long COVID
Several biological mechanisms have been proposed as the potential root causes of long COVID. Currently available approaches to research testing are reviewed, as are potential therapeutics for consideration in experimental medicine studies targeting these mechanisms. These upstream mechanisms may drive one or more physiologic models of long COVID (see Figure 6).

possibly irreversible damage of some of these tissues might explain some of the lingering symptoms of long COVID (see Downstream: Clinical physiology of long COVID, below).

There is evidence linking the level of virus activity during early infection with the subsequent risk of long COVID.[52–54] More virus replication is also associated with more severe acute infection,[70] also a consistent predictor of long COVID. The risk of long COVID appears lower with Omicron variants, which may have lower viral burden than early variants,[71,72] although this may be in part related to pre-existing immunity.[73] The protective effect of vaccines[44,45,47] and antiviral treatment[48,49] provide indirect evidence that virus replication and spread during the acute phase is a major determinant of long-term outcomes.

More direct evidence for the role of virus replication during the acute phase is now emerging. Among hospitalized individuals, nasal viral loads and SARS-CoV-2 RNA detection in blood are associated with long COVID symptoms.[54,74] One study found that outpatients who developed long COVID had a higher nasopharyngeal viral load peak during the first 21 days compared with those reporting full recovery.[52] Others have found that long COVID brain fog is associated with prolonged time to viral clearance.[53,74] Still, even minimally symptomatic acute infection can cause long COVID.[75] Thus, viral replication and systemic spread may be required to initiate the pathways that lead to long COVID but are likely not sufficient. The highly variable host response to the virus, which includes the acute and post-acute activity of both innate and adaptive immune cells, also clearly shapes who gets long COVID.

The best way to prevent long COVID is to prevent the initial infection. Vaccination and natural immunity from prior infections may not completely prevent infection (Figure 5A) but could blunt the initial virus spread and hence limit both acute and chronic sequelae.[44–46]

Randomized studies aimed at treating acute COVID-19 are also an easy place to start. If the development of long COVID is determined by virus replication and tissue tropism, then the rapid and perhaps sustained administration of potent antiviral therapy during acute or early infection should prevent disease. A 5-day course of nirmatrelvir/ritonavir—which might not have optimal tissue penetration—might work to prevent acute symptoms but not long COVID. Sustained administration of more potent drugs, or combinations, might be needed. Presumably, such interventions work by reducing tissue damage and averting many of the mechanisms outlined below.

Unfortunately, the first generation studies of antivirals and immunomodulators for COVID-19 were not designed to address long-term outcomes. Figuring this out retrospectively has been challenging, and none of these studies is likely to lead to regulatory approval. In the US Veterans' Affairs system, the use of nirmatrelvir/ritonavir during acute infection reduced the risk of PASC (including long COVID).[48] More definitive studies, in which people randomized to receive antiviral agents are followed longitudinally, are needed to definitively demonstrate this effect. Promising data on ensitrelvir, a protease inhibitor authorized in Japan, were recently presented.[76] The early data from studies of monoclonal antibodies have been mixed.[76]

In a large placebo-controlled study of metformin, ivermectin, and fluvoxamine, those who received metformin for 2 weeks were less likely than those receiving placebo to be diagnosed with long COVID.[77] The other drugs were not protective. The authors speculated that metformin was protective due to a
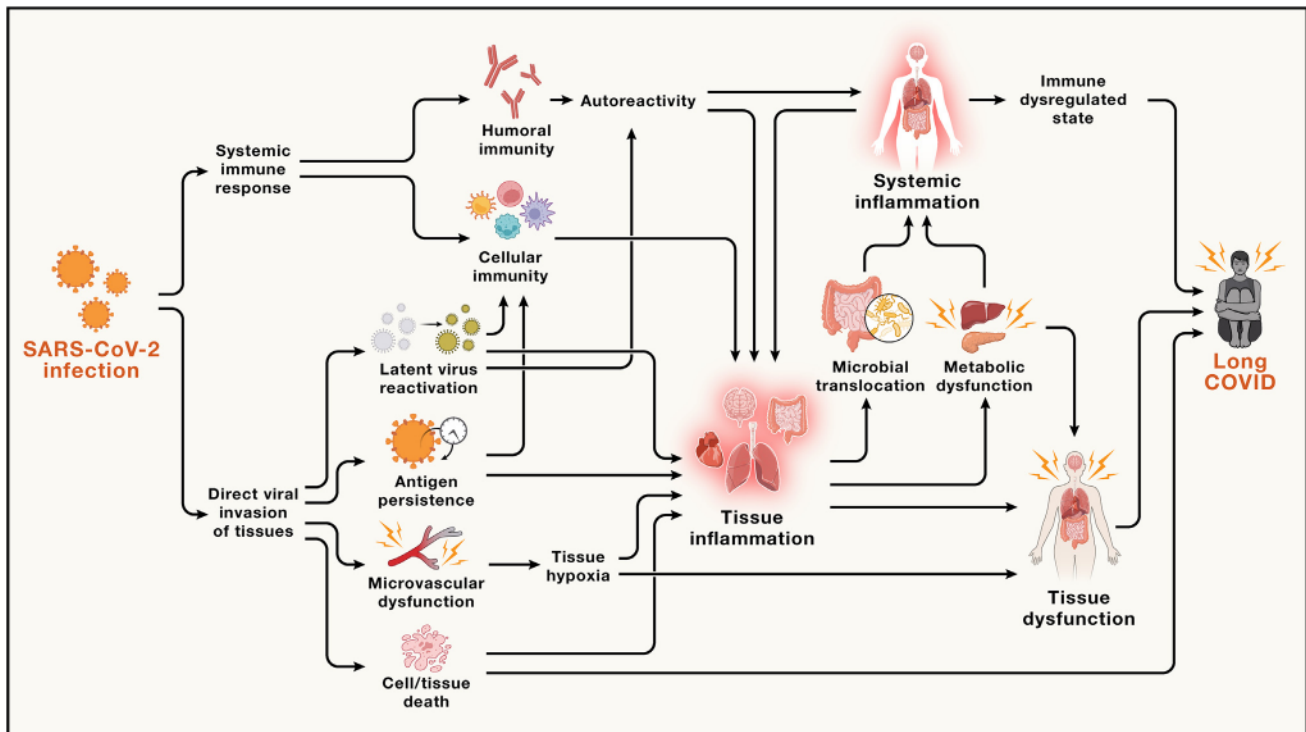
Figure 5 Potential relationships between the biological mechanisms of long COVID
The proposed mechanisms interact in complex ways to ultimately drive downstream physiologic changes that result in long COVID symptoms.

combination of antiviral activity and reductions in oxidative stress and inflammation; it was demonstrated in a follow-up analysis to have reduced the viral load.[78] Currently, there is no evidence that use of metformin beyond the acute period is beneficial. Randomized trials of anticoagulation and antithrombotic therapies during the acute phase have also yielded mixed results.[79–83] Notably in one study, antiplatelet agents but not anticoagulants for acute COVID-19 improved 6-month survival and were associated with improvement in health-related quality of life.[84] Early treatment with certain immunomodulators was also beneficial for the acute disease; though the role of these drugs in preventing long COVID is unknown.[84]
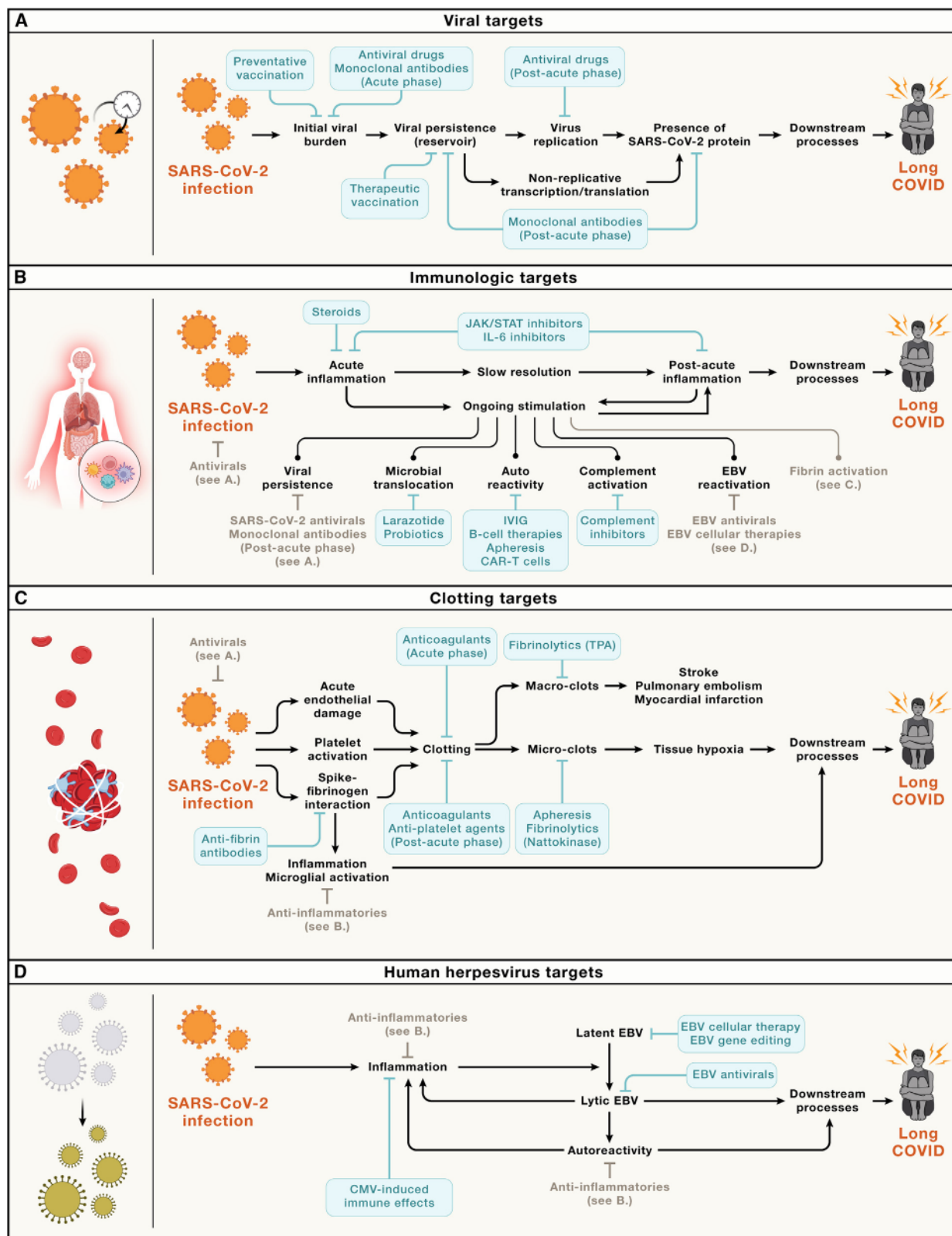
Taken together, these studies suggest that early interventions for COVID-19 could mitigate long COVID and support the pursuit of a robust preventive agenda, which is currently lacking. At the very least, future studies of interventions during acute infection should evaluate post-acute sequelae as a pre-specified outcome. However, it will also be critical to consider testing therapeutic approaches specifically designed to prevent long COVID (even if there is no symptomatic benefit in the acute phase[85]). This would require trials specifically designed and powered around post-acute outcomes, for example of longer-course antiviral treatment during the acute phase of infection with either patient-reported outcomes (e.g., fatigue) or outcomes rooted in the biologic or physiologic abnormalities outlined below.

Post-acute virus persistence
Early in the pandemic, the common assumption was that SARS-CoV-2 infection would prove to be transient, as is the case with

coronaviruses in general. This assumption was challenged by early reports that viral nucleic acid and proteins could be detected in the gut months after infection.[86] Subsequently, reports indicated that some people—both immunocompromised[87–89] and immunocompetent[90–93]—could harbor replicating virus for months. Many more studies have since emerged. A proportion of individuals with long COVID have intermittently detectable antigen in plasma for up to a year post-infection,[94,95] and a transcriptomic analysis demonstrated upregulation of SARS-CoV-2 RNAs in whole blood of people with long COVID.[96] At least one study has noted serologic responses in those with long COVID that are broadly suggestive of chronic antigen stimulation, even when the direct detection of such antigen was limited.[97] We note, however, that not all studies have been positive,[98] and publication bias that may limit reporting of negative results is a potential challenge.

It is possible for RNA viruses to persist for months to years, as demonstrated with Ebola, Zika, and measles[99]; there is also precedence for the persistence of feline coronaviruses.[100,101] Putative mechanisms include immune evasion (e.g., virus-mediated inhibition of interferons) and/or the avoidance of cell death through selection of less virulent viruses.[99] Although the precise localization of SARS-CoV-2 persistence is unknown, there is an emerging consensus that this is a tissue-based process. Autopsy studies[69] and tissue biopsy studies in living individuals[102] have identified viral RNA or protein in various tissues months after the infection was apparently cleared from the nasopharynx. Newborns whose mothers had COVID-19 over 10 weeks prior have been found to have detectable SARS-CoV-2 RNA or spike

A. Viral targets

Preventative vaccination
Antiviral drugs
Monoclonal antibodies (Acute phase)
Antiviral drugs (Post-acute phase)

SARS-CoV-2 infection → Initial viral burden → Viral persistence (reservoir) → Virus replication → Presence of SARS-CoV-2 protein → Downstream processes → Long COVID

Therapeutic vaccination

Non-replicative transcription/translation

Monoclonal antibodies (Post-acute phase)

B. Immunologic targets

Steroids
JAK/STAT inhibitors
IL-6 inhibitors

SARS-CoV-2 infection → Acute inflammation → Slow resolution → Post-acute inflammation → Downstream processes → Long COVID

Ongoing stimulation

Antivirals (see A.)

Viral persistence | Microbial translocation | Auto reactivity | Complement activation | EBV reactivation | Fibrin activation (see C.)

SARS-CoV-2 antivirals
Monoclonal antibodies (Post-acute phase) (see A.)

Larazotide
Probiotics

IVIG
B-cell therapies
Apheresis
CAR-T cells

Complement inhibitors

EBV antivirals
EBV cellular therapies (see D.)

C. Clotting targets

Antivirals (see A.)

Anticoagulants (Acute phase)
Fibrinolytics (TPA)

SARS-CoV-2 infection → Acute endothelial damage / Platelet activation / Spike-fibrinogen interaction → Clotting → Macro-clots → Stroke, Pulmonary embolism, Myocardial infarction

Clotting → Micro-clots → Tissue hypoxia → Downstream processes → Long COVID

Anti-fibrin antibodies

Inflammation Microglial activation

Anticoagulants
Anti-platelet agents (Post-acute phase)

Apheresis
Fibrinolytics (Nattokinase)

Anti-inflammatories (see B.)

D. Human herpesvirus targets

Anti-inflammatories (see B.)

SARS-CoV-2 infection → Inflammation → Latent EBV → Lytic EBV → Autoreactivity → Downstream processes → Long COVID

EBV cellular therapy
EBV gene editing

EBV antivirals

CMV-induced immune effects

Anti-inflammatories (see B.)

legend on next page)

protein in neonatal stool in the first week of life, suggesting *in utero* transmission.[103] SARS-CoV-2 nucleocapsid protein has been found in post-COVID-19 muscle biopsies but not in samples obtained before the pandemic.[104] Fat cells and the endothelium may also be a reservoir.[68,105] But the most straightforward and accessible reservoir is likely to be in the gut[86,89,102]: SARS-CoV-2 RNA is detectable in stool during the early post-acute phase,[106] and persistent spike RNA in gut lamina propria was identified for over 2 years post-infection among individuals with long COVID.[107]

Many questions remain. The levels of viral particles are generally very low, and false positive findings are possible. To address this concern, analysis of samples banked before and during the pandemic (true negative controls)[95] revealed that the prevalence of SARS-CoV-2 antigen detection was significantly higher in post-acute pandemic-era plasma specimens and that antigen detection correlated with the severity of acute illness. This suggests that the signals observed across multiple studies are real. However, it is unclear if these findings reflect an actively replicating virus population, a stable reservoir of virus-producing cells, or remnants of a remote, extinguished infection. Recently, reports of double-stranded SARS-CoV-2 RNA have suggested the presence of active viral life cycling.[96,107] How a small, difficult-to-detect virus population would cause a systemic, disabling disease that lasts for years is unknown. Finally, despite the growing body of evidence,[21] the association between virus persistence and the development of long COVID is modest at best. Studies are often conflicting, and in most cases, the comparison to recovered and pre-pandemic controls has been limited.[108]

If the central problem is persistence of the virus, the optimal solution could be an antiviral strategy. Such studies have been initiated, and more are planned. Antiviral drugs such as nirmatrelvir/ritonavir, remdesivir, or molnupiravir could work by blocking active replication but might not have any effect on a stable, persistent reservoir (Figure 6A).

Given its established role in treating acute infection, most antiviral studies are using standard doses of nirmatrelvir/ritonavir. A randomized, control study of nirmatrelvir/ritonavir versus placebo/ritonavir for 15 days in 155 individuals with long COVID[109] showed no difference in pooled severity of several classic symptoms at week 10. Although disappointing, there are several biological explanations beyond lack of efficacy that could explain this result. The population enrolled could have included too few individuals with virus persistence. Concerns have been raised that tissue penetration, a factor that might be key in eliminating established reservoirs, of this drug is limited. As outlined in an overview of those with experience taking nirma-

trelvir/ritonavir for long COVID,[110] the duration of therapy needed to clear an infection is also unknown and might be far longer than what is currently being studied (maximum 25 days). Finally, the dose selected for blunting acute infection may not be the optimal dose for treating a chronic, resilient infection. Combination regimens of more potent drugs or those targeting multiple points in the virus life cycle administered for a longer period may need to be tested.

Approaches beyond antivirals should also be considered. Virus production in the absence of new infection events could require interventions that target and eliminate the reservoir. Monoclonal antibodies that target the spike protein might be effective in this case, assuming expression of spike protein is causally associated with long COVID and that the antibody has a cytotoxic effector function. These antibodies could have beneficial effects through neutralization of proteins and virions, opsonization of virions and infected cells, and the induction of antibody- or complement-mediated cellular cytotoxicity if viral proteins are expressed on the cell surface.[111] Over the course of the pandemic, variants emerged with mutations in the spike region that eventually resulted in deauthorization of monoclonal antibodies, as they were no longer effective against actively circulating variants. However, theoretically, variants that persist in a person with long COVID are archived and could respond to older monoclonal antibodies, even as the globally circulating viruses are resistant. One provocative report suggested dramatic improvement in those receiving monoclonals for reinfections.[112] With all these caveats, at least one randomized trial of monoclonal antibodies is now underway. More studies, ideally with updated agents with activity against modern variants, will be needed to fully address the question.

Post-acute inflammation

Chronic inflammation has been implicated in many diseases, including cardiovascular disease and cancer.[113–115] COVID-19 is highly inflammatory,[116,117] and it is now apparent that SARS-CoV-2 can lead to a chronic inflammatory state, although this might improve over time.[118] Multiple studies have linked markers of inflammation and immune dysfunction with long COVID. Several efforts have now begun to distinguish an inflammatory subset of long COVID.[119–121]

Multiple studies have also begun to link specific markers of inflammation and immune dysfunction with long COVID. In a prospective study conducted in Germany, interleukin (IL)-1beta, IL-6, and tumor necrosis factor (TNF) (the "macrophage triad") were associated with long COVID.[122] Similar findings have been made by others.[123–129] for example, IL-6, TNF alpha (TNF-), and interferon-gamma-inducible protein 10 (IP-10)

Figure 6 Mechanistic targets of long COVID prevention and treatment
(A) Viral targets. SARS-CoV-2 during both the acute and post-acute phase could be targeted by interventions that reduce spread (vaccination, antiviral drugs, monoclonal antibodies). These agents may also have an effect on virus persistence in the post-acute phase.
(B) Immunologic targets. Agents like steroids and anti-inflammatory drugs may play a role in mitigating inflammation during acute infection. During the post-acute phase, anti-inflammatory drugs targeting certain pathways (e.g., JAK/STAT, inflammasome, and IL-6) may dampen ongoing inflammation. Other therapies might target drivers of inflammation like persistent virus (see A), microbial translocation, autoreactivity, clotting (see C), or EBV reactivation (see D).
(C) Clotting targets. Anticoagulants and antiplatelet agents administered during the acute phase and possibly the post-acute phase may be of benefit, whereas fibrinolytics or apheresis may be considered to reduce the burden of existing clots.
(D) Human herpesvirus targets. Antivirals administered during the acute and post-acute phase of SARS-CoV-2 infection could alter pathology related to active EBV, while novel cellular and gene therapies may be considered to alter pathways related to latent EBV infection.
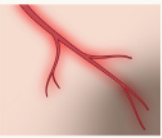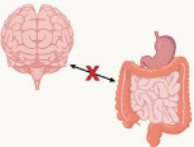
Figure 7 Physiologic models of long COVID
The upstream biological mechanisms (Figure 4) may drive one or more physiologic models of tissue dysfunction in long COVID. These physiologic models could in turn explain many of the symptoms of the condition. Also indicated tests that may be useful and potential therapeutics targeting these physiologic models. Created with BioRender.

were modestly elevated at approximately 2 months in those who developed long COVID as compared with those who did not; these relationships were strengthened when examining neurologic and cardiopulmonary phenotypes.[125–128] Single-cell transcriptomic data have indicated that myeloid/macrophage cells were the likely source of these cytokines.[122] The potential role of inflammatory monocytes and macrophages in long COVID has been the focus of several studies, particularly as these cells might also prove to be a reservoir for persistent infection.[130,131]

The role of chronic T cell activation and dysfunction has received less attention. Several groups have identified chronic activation of a subset of CD8+ T cells among those with long COVID up to 8 months post-infection.[132,133] Although an initial study failed to find substantial differences in T cell responses based on symptomatology at 4 months,[134] cytometry by time of flight (CyTOF) data showed that in comparison to those who fully recovered, those with long COVID had more expression of tissue homing markers on CD4+ T cells and immune exhaustion markers on CD8+ T cells.[135] Similarly, others have identified exhausted T cells in those with long COVID,[136] suggesting chronic immune activation due to factors such as persistent stimulation by SARS-CoV-2 or other antigens.

The activation of mast cells might also play a role in long COVID. Although mast cells are not believed to be infected by SARS-CoV-2, they can be activated indirectly by various external triggers. The systemic production of mediators such as histamine, prostaglandins, and inflammatory proteins can potentially lead to gastrointestinal and pulmonary symptoms. Although high-quality studies of mast cell biology in long COVID are lacking, the similarities between long COVID symptoms and those of mast cell activation syndrome, especially the relapsing-remitting nature of both conditions, have been noted.[137]

To date, most immunology studies have focused on blood measurements. Building upon prior work demonstrating increased metabolic activity in certain tissues following COVID-19,[138–143] radiopharmaceutical agent-based research found tissue-level evidence of activated T lymphocytes in multiple anatomical regions for up to 2.5 years post-COVID-19.[107] There was also evidence of increased T cell activation in spinal cord, gut, and lung among participants with long COVID symptoms. Another study using [11C] PBR28 positron emission tomography (PET) neuroimaging identified ongoing neuroinflammation in several brain regions, which was tied to serologic markers of vascular dysfunction.[144] Such studies begin to connect these biological mechanisms to the physiologic dysfunction, based in tissues, outlined below. More studies evaluating tissue parameters longitudinally would be helpful, but their interpretation is now complicated by frequent SARS-CoV-2 vaccination and reinfection.

Nearly all mechanistic pathways converge on an inflammatory response, either systemically or localized in the tissues. This inflammation can cause tissue dysfunction and physiologic stress responses.[145] Inflammation can also contribute to immune dysfunction and hence inability to clear residual virus, setting up a vicious cycle similar to that in HIV and cancer. If this is the case, blunting the inflammatory response may allow for its root cause to be addressed (Figure 6B).

The development of immunotherapies that target specific pathways in cancer, autoimmunity, transplantation, and other areas of medicine has been one of the greatest recent achievements in medicine. Many of these therapies target pathways that have been associated with long COVID. These include drugs—mainly monoclonal antibodies—that target IL-1beta, TNF-, interferons, and IL-6, among others. Some anecdotal experience with these therapies has emerged,[146] and discussion about early-phase testing is ongoing.

Choosing which immunotherapies to move into the clinic is challenging. Important factors to consider include the specificity,

potency, and duration of therapy needed to see a meaningful response. Nonspecific anti-inflammatory therapies such as corticosteroids reduce the rate of disease progression among patients with severe COVID-19.[147,148] While potent, these agents are associated with potential harm, especially if administered over the long term. Interventions that precisely target a single pathway such as IL-1beta might be insufficient to reverse the broad inflammatory response in long COVID or might simply result in counter-regulatory responses that are equally harmful. For these reasons, consideration should be given to the investigation of more upstream therapies aimed at modulating various arms of the immune response known to be important in acute COVID-19. For example, baricitinib, a US Food and Drug Administration (FDA)-approved immunomodulator for COVID-19, is a Janus kinase (JAK) inhibitor that was initially approved for rheumatoid arthritis and has demonstrated efficacy for acute COVID-19.[149,150] This agent targets the STAT3 pathway, which viruses might use to reduce antiviral immune responses through downregulation of interferons or upregulation of IL-6, IL-10, TNF- , and IL-1B.[151] A multi-center study of baricitinib is planned.

Drugs like rapamycin, an immunomodulator that works via multiple pathways related to protein regulation, autophagy, and cellular senescence,[152] may also warrant testing; a trial of this agent in CFS is underway. Consideration of low doses will be important, given the narrow therapeutic window.

Inhibiting the inflammasome is also attractive. Inflammasome-specific therapies would be expected to limit the activation of downstream cytokines and interferons, blunting the inflammatory response without suppressing the immune system.[153] An example is colchicine, but other, more targeted therapies exist. Such agents are likely to have a milder side-effect profile and could be attractive for long-term treatment. Other approaches, such as IL-6 antagonism with tocilizumab or sarulimab,[154,155] particularly for those with high levels of circulating cytokines, might also be deserving of further study. If immune exhaustion due to persistent antigen stimulation is indeed confirmed to be a driver of long COVID, the use of checkpoint inhibitors might be considered in carefully designed studies to improve the ability to clear viral reservoirs. Similarly, if deficiencies in coordination between the innate and adaptive arms of the immune system are identified, certain treatments, such as IL-15 agonism, which can improve cross-talk between natural killer (NK) and T cells, may be considered, as is being done in HIV infection.

B cell responses and autoimmunity

Severe COVID-19 is associated with autoimmunity,[156–158] and autoantibodies have been observed in people hospitalized with COVID-19.[157] Whether autoantibodies are a cause or consequence of severe COVID-19 is complicated. Antibodies to interferon that are thought to pre-date the infection, for example, can lead to severe manifestations of COVID-19.[159–162] Acute infection can also induce autoantibody production.[163–165] COVID-19 is also associated with an increased incidence of autoimmune medical conditions such as Sjogren's syndrome, rheumatoid arthritis, lupus, and inflammatory bowel disease.[166,167]

The data are much more mixed regarding the relationship between autoimmunity and long COVID. Some studies have identified a high prevalence of antinuclear antibodies[74,168,169] and others a low prevalence consistent with that in the general population.[122,170,171] In conditions such as systemic lupus erythematosus (SLE), antinuclear antibodies are characterized by multiple subtypes, variability in expression based on factors including disease stage and patient ancestry.[172] Even when present, antinuclear antibodies are not necessarily pathogenic, further complicating the interpretation of these studies in long COVID.

Despite the clear presence of a post-COVID-19 autoreactivity signature[173] and the potential for molecular mimicry between SARS-CoV-2 spike protein and host proteins,[174] two studies failed to identify shared autoantigen signatures of long COVID using complementary technologies and multiple case definitions.[137,173] These studies, while comprehensive, have limitations. For example, because phage immunoprecipitation sequencing (PhIP-Seq) screens against linear peptides, the methodology fails to detect autoreactivity related to protein conformation or post-translational modification. These technologies also do not assess whether autoreactive cellular immunity could be at play. It is also possible that there is no shared signature but that autoimmunity is still important on an individual level. Paradoxically, another study identified that certain anti-chemokine antibodies against CCL21, CXCL13, and CXCL16 negatively correlated with long COVID.[175] Although the reason for this was unclear, it is possible that their presence modulates persistent immune activity following COVID-19.

More recently, data have begun to emerge mechanistically tying long COVID to potential autoimmune processes. In one provocative study, total immunoglobulin G (IgG) isolated from people with different long COVID phenotypes was transferred into mice,[176] which in some cases caused heightened pain sensitivity and abnormal movements. In another study,[177] total IgG from individuals with neurologic long COVID symptoms was found to react with tissue from the human pons as well as several mouse tissues of the central and peripheral nervous systems. When injected into mice, researchers similarly observed impaired coordination, elevated pain sensitivity, and muscle weakness. In both studies, the authors concluded that antibodies directed at tissues might be the cause of long COVID.

Thus, following a compelling story in the acute setting, progress is now being made in defining the role of autoimmunity and autoantibodies in the chronic disease state. If these studies can be replicated, targeting the autoimmune response may prove beneficial in long COVID.

Developing therapeutics that target autoantibodies—even if their specificity is unknown—remains a high priority. Several approaches are possible (Figure 6B). In systemic lupus erythematosus, rheumatoid arthritis, and multiple sclerosis, broadly acting approaches that reduce inflammation (steroids, methotrexate, dimethyl fumarate) and those that block the production of all antibodies (anti-CD20 antibodies such as rituximab) are often used.[178] Genetic therapies such as CAR-T cells that target all B cells or a specific subset are now being developed for SLE and other autoimmune disorders[179] and might eventually be considered for long COVID. The data are insufficient to justify large-scale testing of these interventions now, given their costs and risks, but this could change should data emerge clearly linking consistent autoimmune responses to an endotype of long COVID.

Plasmapheresis, by which antibodies are removed from the plasma and then blood returned to the body, is used in certain autoimmune hematologic disorders, including vasculitis and thrombotic thrombocytopenic purpura. Randomized studies of plasmapheresis in long COVID are underway, although it is not clear whether such a treatment approach could be scalable.

One therapy for which there is some anecdotal experience is intravenous immunoglobulin (IVIG). IVIG is used to treat various autoimmune disorders, including immune-mediated thrombocytopenia, Kawasaki disease, and Guillain-Barre syndrome. Although the mechanism is unclear, a leading theory is that the Fc portion of the immunoglobulins competes with or inhibits the Fc portion of the disease-causing autoantibody.[180,181] A subset of antibodies in IVIG may also suppress cytokine production or neutralize circulating cytokines, directly bind circulating autoantibodies, alter regulatory T cell function, and interact with the complement system.[182–184] Several of these targets may be helpful in long COVID, although the treatment is costly and of limited availability. Randomized studies of IVIG in long COVID have begun, including one occurring via the NIH RECOVER Initiative. If successful, understanding the mechanism by which this agent provides benefit could be helpful in developing more specific therapies targeting these same mechanisms, which could be made more widely available than IVIG.

Thrombotic events and microclots

Acute SARS-CoV-2 infection can result in a hypercoagulable state and a high risk of thromboembolic events.[185–187] Although the risk is higher in severe disease,[2] this can occur in minimally symptomatic patients, including previously healthy adults.[188] The mechanism is unknown but may be related to endothelial damage as well as generation of prothrombotic autoantibodies including those against fibrinogen.[156,189] Antiphospholipid antibodies, which would be expected to result in hypercoagulation, have been identified in patients with COVID-19 and are associated with activation of neutrophils and coagulation pathways.[156] As other acute viral infections generally do not cause coagulopathies, something unique to SARS-CoV-2 may be required to initiate the clotting cascade.

One study found that the spike protein binds fibrinogen, resulting in conformational changes in this protein.[189] In a mouse model, this spike protein-fibrinogen interaction results in the formation of structurally abnormal clots that are resistant to plasmin degradation; these aggregates are able to directly induce microglial activation in the brain and hence, in theory, might contribute to neurocognitive long COVID.[189] Similar pathways have been implicated in the development of Alzheimer's disease: fibrinogen can induce neuronal spine elimination and promote cognitive deficits through pathways mediated by CD11b-CD18 microglial activation.[190] Blocking these pathways reduced the deficits, suggesting a direct link between fibrin dysregulation and immune-mediated neurodegeneration.[191] In hospitalized individuals, fibrinogen levels during the acute phase of infection predict both subjective and objective neurocognitive performance at 6 and 12 months.[192]

Multiple lines of investigation are emerging that implicate clotting as a mechanism for long COVID. Some studies have identified circulating aggregates of platelets and clotting proteins ("microclots") in people with long COVID.[193,194] These microclots are resistant to fibrinolysis and associated with inflammatory molecules,[193–195] but the findings remain to be confirmed in independent cohorts. If they are indeed specific to long COVID, an imbalance between clot generation and degradation may be central to the syndrome and could have consequences related to alterations in tissue oxygen supply that could result in organ dysfunction and thus long COVID symptoms (see physiology of long COVID, below). Another study found an increased von Willebrand factor (vWF)/ADAMST13 ratio among people with long COVID at 6 months after their acute infection, creating a prothrombotic and potentially hemolytic state that may also activate the complement pathway.[196] The same study found increased macrophage-platelet aggregation in people with long COVID.

There is a strong rationale to develop therapies that target the coagulation system, platelet aggregation, and the specific nature of the microclots that have been reported to date (Figure 6C). In an uncontrolled series of 24 individuals, combined antiplatelet (aspirin, clopidogrel) and anticoagulant therapy (apixiban) appeared to confer benefit in those with microclots.[195] The authors reported that all participants experienced symptom resolution in association with reduction in microclot burden. This observation is provocative, but controlled studies are needed before it is pursued further. Importantly, antiplatelet and anticoagulation therapies prevent the formation or expansion of clots but are unable to eliminate them once formed. This approach may work as the root cause of the disease (e.g., virus persistence) is treated or slowly wanes. Indefinite antiplatelet and anticoagulation therapy for all people affected by long COVID will pose concerns given their inherent risks (e.g., bleeding, etc.).

If microclots need to be removed, attempts to accelerate fibrinolysis may be warranted. While medications used in clinical care of large-vessel occlusions such as pulmonary embolism or stroke (e.g., tissue plasminogen activator and streptokinase) have an unacceptable risk profile, other treatments may be safer. Nattokinase is an oral agent derived from a bacterium present during soybean fermentation that promotes degradation of fibrin.[197] While it has not been studied in a controlled manner, it is at least somewhat bioavailable[198] and has become popular among some patients with long COVID. No studies are currently registered on clinicaltrials.gov.

Eventually, more targeted mechanism-specific therapies may be needed. The observation that the interaction between spike protein and fibrin may be the fundamental driver of clotting pathology in acute COVID-19 suggests that this same mechanism may be responsible for clotting pathology in long COVID. If so, it may represent a therapeutic target either through clearance of spike protein (as above) or by targeting the abnormal fibrin itself, as suggested in a mouse model.[189] Similarly, therapies addressing inflammation by targeting complement pathways could be trialed.

Reactivation of latent viral infections

Epstein-Barr virus (EBV) is ubiquitous, with over 90% of adults having been exposed.[199] After the acute infection resolves, EBV persists indefinitely in a latent episomal form in memory B cells. Under certain conditions, including the stress response to other infections, EBV can reactivate and enter the lytic phase. When activated, EBV expresses viral genes and produces infectious progeny, resulting in cell death. This process may be

asymptomatic or result in symptoms like those associated with long COVID.

While some studies have assessed EBV genomes in blood, a more typical approach is to utilize the characteristics of antibody responses against EBV to detect reactivation. One study found a link between early antigen D (EA-D) IgG responses—a putative marker of recent EBV reactivation—and post-COVID-19 fatigue.[200] In addition, the study identified high-level Epstein-Barr nuclear antigen 1 (EBNA1) IgG responses—a putative marker of total EBV burden—in those with neurocognitive symptoms.[200] Another study found an association between EBV reactivation, cardiopulmonary symptoms, and reduced performance on cardiopulmonary exercise testing (CPET).[201] Other groups have found that EBV reactivation during acute SARS-CoV-2 infection is associated with increased risk of long COVID symptoms[74] and that the magnitude of EBV EA-D responses correlated with the number of symptoms reported.[202] These findings were recently extended in a study demonstrating higher antibody reactivity against specific EBV antigens, in particular those on the surface of virions, in the absence of viremia.[136] Long COVID has been reported in people who had no evidence of prior EBV infection, however. This might reflect misclassification of the diagnosis or the fact that multiple mechanisms (EBV and others) can lead to the development of long COVID.

The mechanism for the remarkably consistent association between SARS-CoV-2 and EBV remains to be defined. While the presence of genomes from or immune responses to EBV and other herpesviruses (e.g., human herpesvirus 6, HHV-6) have been associated with ME/CFS, the role of these pathogens is poorly understood.[203–205] However, the recent discovery of the potentially causal role of EBV in multiple sclerosis[206] through mechanisms such as molecular mimicry[207] suggests that relationships between EBV and some of the autoimmune processes outlined above may exist.

If reactivation of EBV contributes to long COVID, then antiviral agents aimed at this co-pathogen may need to be given at the time of reactivation, which is expected to coincide with acute SARS-CoV-2 infection (Figure 5D). However, there are no potent EBV-specific drugs currently available. High-dose acyclovir, valacyclovir, ganciclovir, and maribavir, among other drug candidates, all have modest anti-EBV activity *in vitro* but have not been developed for EBV in the clinic.[208] It is unclear whether EBV antivirals beyond the period of reactivation would help. Interest in this approach stems from proof-of-concept data in ME/CFS,[203] where some studies showed symptomatic improvement allowing return to usual activities,[209] as well as improvement in fatigue and cognitive function.[210,211]

It is also possible that immunotherapies aimed at enhancing clearance or blocking harmful immune pathways will be helpful. Cellular therapies (for example, targeting EBV-infected B lymphocytes and plasma cells) are under investigation for multiple sclerosis. If a causal role of EBV is proven for long COVID, similar strategies might also be pursued.

Alterations in the microbiome and microbial translocation

Acute COVID-19 can cause severe and long-lasting disruption of the gut microbiome,[212] in part due to the high burden of ACE2 receptors in the gastrointestinal tract.[213] This perhaps has an effect on beneficial commensal organisms.[214] Some studies show that microbiome changes are associated with long COVID.[215,216] Recent work has identified differences in the diversity of the microbiome and its associated metabolic pathways in people with ME/CFS compared with healthy controls.[217,218]

SARS-CoV-2 infection of gastrointestinal epithelial cells can result in tissue inflammation, cell death, and harm to the mucosal barriers in the gut and other tissues. In some cases, this can lead to systemic exposure to bacteria and other organisms. Zonulin, a protein that reduces tight junction permeability, rises after acute infection and is associated with more rapid COVID-19 progression.[219,220] Zonulin is also elevated in multisystem inflammatory syndrome (MIS-C), a severe post-COVID-19 illness in children.[221] Elevated levels of zonulin, beta-D-glucan (a fungal cell wall product), and lipopolysaccharide binding protein (a marker of bacterial translocation) were found in the blood of those with long COVID, in comparison to individuals who fully recovered.[222] These products were associated with levels of inflammatory cytokines, including IL-6 and TNF- . This is consistent with prolonged or perhaps irreversible damage to mucosal surfaces in the gut and perhaps elsewhere.

There are several trials underway investigating the impact of probiotics and at least one of fecal microbiota transplantation. Agents to alter the intestinal flora and promote epithelial healing, such as probiotics or rifaximin, might also be considered.[223] In a large clinical trial conducted in Hong Kong, people with long COVID were randomized to a synbiotic preparation or placebo and followed prospectively.[224] Those who received the active intervention reported a higher degree of resolution of multiple long COVID symptoms, suggesting that modulation of the gut microbiome could be beneficial.

The zonulin antagonist AT1001 (larazotide acetate) has shown promise in a child with severe COVID-19 who developed MIS-C refractory to anti-inflammatory therapy. Larger trials for MIS-C are now underway. Should similar mechanisms be at play in long COVID, a trial of this or other agents meant to restore gut barrier integrity could be of benefit (Figure 6B). Other potential therapeutics targeting B-D-glucan and pathways tied to fungal translocation, such as piceatannol, would likely need to be developed pre-clinically but could provide further insight into the biology.[222]

Mitochondrial dysfunction and metabolic derangements

SARS-CoV-2 infection alters mitochondrial structure and function through mitochondrial protein binding and effects on mitochondrial gene expression, with differential effects across tissue types.[225] Long COVID has been tied to metabolic perturbations, including dysfunction of mitochondria. An early study found abnormalities in levels of mitochondrial proteins in neural-derived exosomes of patients with neuropsychiatric long COVID in comparison to pre-pandemic controls and recovered individuals.[226] Others have observed elevated lactic acid levels and alterations in fatty acid oxidation.[227,228] Using metabolomics, a study showed that individuals with long COVID exhibit disturbances in the resting metabolic state,[229] indicating altered impaired pyruvate/lactate metabolism. Whether these alterations in mitochondrial health begin with the acute infection

and how they change over time remains unclear. However, another group assessed changes in SARS-CoV-2 RNA levels in both animal and human models with alterations in expression of host genes related to mitochondrial function and the integrated stress response (ISR). SARS-CoV-2 was found to reduce expression of genes associated with mitochondrial oxidative phosphorylation, and in autopsy specimens, mitochondrial gene expression continued to be impaired in several tissues, including the heart.[225] They suggested that ongoing inhibition of oxidative phosphorylation pathways could contribute to long COVID symptoms. Another study in the Netherlands, in which muscle biopsies were performed in individuals with long COVID who reported prominent post-exertional malaise (PEM) symptoms (a classic long COVID symptom), found metabolic alterations and reductions in mitochondrial function characterized by lower capacity for oxidative phosphorylation.[105]

Mitochondrial pathology is intertwined with several of the potential mechanisms of long COVID. Supplements like N-acetylcysteine have shown promise in small, uncontrolled studies. One early-phase trial of AXA1125, a combination of five amino acids and N-acetylcysteine, showed some benefit.[230] Although the 1:1 randomized study did not achieve its primary endpoint of improvement in measures of mitochondrial respiration, treatment with this agent was associated with significant reductions in self-reported long COVID fatigue.

DOWNSTREAM: CLINICAL PHYSIOLOGY OF LONG COVID

The mechanisms described above address how SARS-CoV-2 initiates the process but do not provide an explanation for how long COVID symptoms result and how health is affected. The mechanisms by which these pathways drive abnormal physiology will ultimately determine how long COVID is diagnosed, managed, and treated. Here, we review several possible pathophysiologic models—the organ system impact of the above intertwined biological pathways—that might explain long COVID (Figure 7). We also review potential therapeutic targets.

End-organ tissue damage

The most efficient explanation for long COVID is direct virus-mediated damage of tissues that was initiated during the preceding infection and lingers well beyond the acute phase. Post-COVID-19 pulmonary fibrosis is a classic example of irreversible tissue damage that results in chronic symptoms; less dramatic irreversible tissue damage—potentially disproportionate to the severity of acute infection or not detectable with current measurements—may also be at play. SARS-CoV-2 infection of the pancreas has been implicated in the development of post-COVID-19 diabetes (another type of PASC), providing additional proof of principle.[231–234] Direct tissue damage can also account for excess risk of thromboembolic events (e.g., stroke, pulmonary embolism, and MI) and hepatobiliary disease.[235] Damage to the olfactory system leads to anosmia (a common and unique feature of COVID-19), and abnormalities in these neurons can track to vulnerable regions in the brain in which structural changes, presumably sequelae of acute infection, have been found.[236] While sustained neurologic or glial injury has not been observed, initial direct viral-mediated damage to these tissues may result in long-term effects to the brain or peripheral nervous system.[126,128,237–240]

If irreversible tissue damage and dysfunction are really the cause of long COVID, then treatment of established disease will rely upon supportive care, symptom management, and non-pharmacologic therapy to improve function. A detailed summary of the many non-pharmacologic approaches for long COVID is beyond the scope of this review. However, many studies of physical and cognitive rehabilitation are ongoing, and rehabilitation is an important part of clinical care for many patients with long COVID. One study demonstrated that a structured physical and mental rehabilitation program resulted in sustained improvement across several domains of physical health, mental health, and quality of life.[241]

Interventions focused on physical rehabilitation have not been without controversy, as certain approaches such as graded exercise have been met with concern because of their potential harm in those with PEM.[242] Organizations focused on rehabilitation have published guidelines with these considerations in mind. A one-size-fits-all model should not apply; not all patients experience PEM, and there may be subsets of patients who could benefit from such therapy, which should be carefully studied.

It is also possible that damage might emerge even before the infection can be diagnosed and treated. Drugs that regenerate damaged tissue could also be helpful. Along these lines, anti-fibrotic agents under investigation for pulmonary fibrosis might be of benefit in those developing this syndrome post-COVID-19. Growth factors that regenerate gut and other tissues might also be worth studying.

Endotheliopathy thromboinflammation and tissue hypoxia

Endothelial dysfunction has been proposed as one component of a unified pathway of long COVID.[243] Multiple mechanisms might lead to inflammation of the endothelium lining the vasculature (endotheliitis) and microvascular disease, and end-organ consequences of this process could lead to long COVID.

During acute and presumably persistent infection (if it occurs), SARS-CoV-2 binds endothelial cells, leading to alterations in their function. As outlined above, SARS-CoV-2 can activate platelets and bind fibrinogen, generating clots. The resulting localized damage can enhance the inflammatory state, leading to a vicious cycle of tissue harm and clotting. The release of various cytokines can also lead to the formation of neutrophil extracellular traps (NETs), which contribute further to thrombosis. Studies have shown persistent elevations in vWF and cytokines reflecting vascular injury and repair in the months following COVID-19.[244,245] A systemic proteomic study in people with and without long COVID revealed that those with persistent long COVID had persistent evidence of both activation of the classical complement system and the maintenance of a hypercoagulable state. These and other abnormalities resulted in chronic thromboinflammation and endothelial dysfunction.[196] Another study using dynamic contrast-enhanced MRI demonstrated disruption of specific regions (temporal lobes, frontal cortex) of the blood-brain barrier, which is comprised of endothelial cells supported by various glial cell components of the

nervous system, in those with neurocognitive symptoms but not in those without these symptoms.[246]

It is easy to construct disease models in which endotheliitis and microclots reduce tissue perfusion, leading to end-organ dysfunction. Some studies have found hypoperfusion using CPET,[247] arterial spin label MRI of the brain,[248] and retinal vascular examination.[249] Small-fiber neurons are particularly susceptible to hypoxemia, and transient or permanent harm to this system can contribute to pain, dysautonomia, and other classic symptoms of long COVID (see below). In one provocative study of patients with and without severe PEM, study participants were asked to induce their symptoms via a strenuous exercise protocol.[104] Those with PEM/long COVID had at baseline a lower exercise capacity (defined by VO_2max), a higher density of amyloid-containing deposits in muscle biopsies, and more focal muscle necrosis, among other abnormalities. Exercise induced a higher density of these deposits, although there was no evidence that the deposits were associated with local tissue hypoxia/damage.

For microvascular disease to cause long COVID, one must assume that the inciting factor (SARS-CoV-2) persists for months or that once initiated, the process can persist even in the absence of the virus. No study has yet made the link from any of these pathways (endotheliitis, thromboinflammation, microclots) with tissue damage, but there is enough evidence to justify deeper investigations.

Long COVID is a systemic disease. As any tissue might be affected by microclots, amyloid deposits, and/or endotheliitis, developing therapeutics to prevent or reverse these abnormalities should be a high priority. Such therapies would need to prevent clotting (discussed above) or stabilize, protect, and repair endothelial tissue. Models of atherosclerosis, for which endothelial dysfunction driven by cholesterol is one of the earliest manifestations, are potentially helpful.[250] Statins have pleiotropic effects and have been shown to benefit those with HIV infection and low-to-moderate cardiovascular risk factors.[251] These agents are widely available and safe, and studies are underway. Metformin may also have a similar effect and warrant further study in the post-acute phase.[252] Other agents affecting endothelial reactivity through alteration of various pathways that alter vasoconstriction and vasodilation—ACE inhibitors, antioxidants, beta blockers, calcium channel blockers, and phosphodiesterase inhibitors—could also be studied.[253] Drugs like ivabradine (discussed below) may also affect endothelial function, even though this is not its primary mechanism of action.[254]

Neuropathy and dysautonomia

The nervous system includes small fibers that innervate all tissues and transmit motor and sensory signals to maintain homeostatic function. Damage to sensory fibers causes the classic neuropathic pain syndromes associated with diabetes mellitus, HIV infection, and certain classes of medications. Autonomic fibers convey motor information in the absence of sensory signals and therefore can cause end-organ dysfunction without classic neuropathic abnormalities. A model by which the multisystem nature of long COVID is explained by disruption of these fibers has been proposed. Direct damage to neurons by the virus or indirect damage related to local tissue injury leads to nerve dam-

age and consequently alters vascular tone, causing systemic hypoperfusion, orthostatic intolerance, and dysregulation of respiratory and/or gastrointestinal function. Effects on the sympathetic and parasympathetic nervous systems can result in loss of homeostatic maintenance of heart rate and contractility. These autonomic disturbances can be perceived as fatigue, cognitive dysfunction, exercise intolerance, and gastroparesis. In cases in which sensory fibers are affected, chronic pain may be prominent. Damage to neurons can take months to resolve, tracking the slow return to health experienced by many with long COVID. When severe, damage can be irreversible and symptoms potentially life-long.

Perhaps the best-characterized syndrome related to dysautonomia is postural orthostatic tachycardia syndrome (POTS). POTS (in adults) is characterized by an increase in the heart rate of 30 more beats per minute without a drop in blood pressure (as one goes from a supine to upright position). Other related syndromes include orthostatic hypotension (and unexplained syncope), which may be due to disruption of the baroreflex pathways. POTS has been reported in 2%–79% of people post-COVID-19,[255–257] which reflects referral bias in some studies. POTS has also been associated with other IACCs, including following the original SARS pandemic.[258]

Many mechanisms, including inflammation and tissue hypoxia (which cause small-fiber neuropathy) and autoimmunity (which can damage multiple components of the neurologic and cardiovascular systems), are believed to be causally associated with POTS. How POTS physiology contributes to the myriad of long COVID symptoms is less well understood. Systemic hypoperfusion due to a poorly regulated response can lead to lightheadedness and potentially feelings of unsteadiness or brain fog. Sympathetic activation can lead to increased heart rate and respiratory rate, which may be perceived as palpitations or shortness of breath.

Compared with uninfected controls, survivors of severe COVID-19 have elevated muscle sympathetic nerve activity that is correlated with reduced exercise capacity.[259] CPET studies found that in comparison to those who fully recovered, a higher proportion of symptomatic long COVID patients had reduced exercise capacity and lower peak oxygen consumption (VO_2).[201] The high proportion with chronotropic incompetence—the inability to mount an appropriate heart rate response to exercise—was consistent with other studies.[260–263] Altered autonomic function and related sinus node remodeling or microcirculatory dysfunction are potential explanations. A small Brazilian study using MIBG found evidence of myocardial sympathetic denervation.[264] Preload failure, impaired peripheral oxygen extraction, and disordered breathing have been observed in other studies using CPET.[247,262,265,266]

While damaged small nerve fibers will not be repaired or regenerated with medical treatment, the downstream effects can be treated. POTS is unique among the long COVID syndromes in that there are effective therapies. These include volume expansion (salt tablets), compression stockings, fludrocortisone (which increases vascular volume), and low-dose beta blockers, among other drugs. Perhaps most promising is ivabradine, a pure heart rate-lowering drug that blocks electrical currents in the sinoatrial node through a selective effect on certain ion

channels (specifically, the I_{funny} channel of the sinoatrial node)[254]; its mechanism of action is independent of contractility and blood pressure. Ivabradine has demonstrated benefit in heart rate and quality of life in small studies of patients with hyperadrenergic POTS.[267,268] A larger study of ivabradine is now underway via the RECOVER initiative, and more are planned.

Disruption of the gut-brain axis

The gut-brain axis is a bidirectional communication network connecting the central and autonomic nervous systems. One example is the hypothalamic-pituitary-adrenal (HPA) axis, a key part of the body's response to stress.[269] Alterations in the HPA axis in long COVID have been suggested.[136] The HPA axis is influenced by enteric microbiota and is capable of modulating immune responses, pain, and higher-order cognitive and emotional functions. As outlined above, SARS-CoV-2 potentially affects this system by altering the balance of beneficial and pathogenic microbes, causing inflammation or disruption of the gut mucosa. This in turn potentially alters neurochemical signaling via pathways originating in the gut.[270]

Disruption of the gut-brain axis allows for a direct connection between post-COVID-19 biologic processes and disruption of normal physiology including autonomic dysfunction, brain fog, and abnormal stress responses (low cortisol) observed in some studies.[136] Recently, evidence has emerged describing the disruption of serotonin neurotransmitter pathways, which in turn can have downstream effects on the vagus nerve and hippocampal activation and result in cognitive changes.[270] This is a potential explanation for brain fog symptoms in general, as well as striking reports of short-term memory deficits among some patients long COVID that we see in clinical practice. Similar pathways have been implicated in other disease states such as ME/CFS.[271]

One framework for understanding IACCs is so-called "sickness behavior"—a protective evolutionary response in which an insult such as infection leads to behavioral changes meant to preserve available energy and direct it toward correcting the underlying pathophysiology (reduced physical and mental activities, reduced caloric consumption, reduced reproductive capacity).[145] In conditions like ME/CFS, the insult and its associated response persist, resulting in an ongoing protective response that is unable to be turned off. The persistence of these sickness symptoms could be due to both systemic or CNS inflammation, driven by some of the processes (e.g., altered afferent vagal nerve inputs[272]) outlined above.

Studies of serotonergic agents like fluvoxamine are underway. Amino acid supplementation or selective serotonin reuptake agents could potentially be beneficial. Glucagon-like peptide 1 receptor agonists might also be considered. These agents, developed for diabetes and obesity, are capable of dampening systemic inflammation via pathways mediated by central nervous system receptors.[273] Their pleiotropic effects across a variety of organ systems are only beginning to be understood.[274]

Modulation of vagus nerve signaling with devices like vagus nerve stimulators (VNSs) has proven effective in depression, and one mechanistic explanation for this is that stimulation of afferent fibers from the GI tract to the brain plays a role.[275] A number of studies of vagus nerve stimulation are currently underway in long COVID, and early studies have suggested that this may benefit a subset of individuals.[276]

Primary neurocognitive dysfunction

One of the most striking and debilitating manifestations of long COVID is problems with attention, memory, and concentration—so-called "brain fog." Similar symptoms are well-described in other IACCs and in cancer patients following chemotherapy. Multiple of the above mechanisms might converge in a set of symptoms experienced as brain fog.

In a series of provocative experiments, one team found that mild COVID-19 limited to the respiratory tract resulted in neurologic inflammation, primarily driven by microglial activation.[277] Since early in the pandemic, many groups observed brain fog as a prominent symptom in non-hospitalized patients.[278–281] Some individuals, particularly those with neurocognitive risk factors such as diabetes, substance use, and learning and attention disorders, appear to have a predilection for developing this condition,[282] suggesting that some people might be selectively vulnerable. Cerebrospinal fluid (CSF) studies have shown that those with this symptom are more likely to have abnormal CSF measurements of protein, white blood cells, or the presence of oligoclonal bands, which in most cases are matched between spinal fluid and plasma.[282] In addition to the structural brain changes and potential perfusion deficits described above,[236] some studies have identified alterations in cerebral white matter on diffusion-weighted MRI, suggestive of inflammation.[283] One study found evidence of a volume shift from extra-neurite (membrane-enclosed) spaces in the brain to the "free-water" space using multi-compartment diffusion microstructure imaging. This shift in volume, particularly at the thalamus and mesiotemporal areas, was associated with impaired cognitive performance.[284] Multiple studies have suggested a role of microglia, resident macrophages of the CNS that interact with neurons and astrocytes.[285,286] Dysregulated microglia may play a central role in altering synapses, disrupting myelination, and even killing neurons or oligodendrocytes.[287] Cytokines observed in studies of long COVID (e.g., IL-6) can be produced by microglia,[126] and fibrinogen dysregulation is also capable of activating microglia,[189,190] suggesting that these cells may play a central role in neurocognitive manifestations of long COVID.

If neurocognitive dysfunction is the primary cause of long COVID, the underlying insult needs to be addressed. Given the lack of clarity regarding the mechanism for this important long COVID endotype, most CNS-focused therapies address symptoms rather than the cause. This includes non-pharmacologic interventions targeting neurocognitive dysfunction, such as cognitive training focused on improving neuroplasticity. Other interventions such as transcranial direct current stimulation (tDCS), which has demonstrated efficacy via mechanisms that may involve improvement in neuronal activity in other conditions associated with cognitive dysfunction,[287–289] are planned by the NIH RECOVER Initiative.

THE PATH TO CLINICAL TRIALS

Above, we have described the current understanding of the pathobiology and pathophysiology that might drive long

COVID. Numerous therapeutic targets have been identified. Nevertheless, the rollout of long COVID clinical trials has been painfully slow, with too few trials overall and remarkably few targeting the proposed mechanisms.[290] This is now beginning to change, with well-powered randomized clinical trials now being reported. Patients and clinicians are still frustrated, however, particularly given the massive successes in vaccines and therapeutics for acute COVID-19. Below, we outline what we believe to be key considerations as we move down the path toward therapeutics for long COVID.

The role of sex

As with other IACCs, women, particularly pre-menopausal women, are more likely than men to have long COVID.[1,2,18,291,292] Studies have begun to suggest several sex-based biological differences in immune responses (higher anti-EBV responses, higher frequencies of cytolytic, tissue homing T cell subsets),[135,293] which can be broadly summarized as a general exaggeration of both innate and adaptive immune responses in women compared with men. Importantly, similar responses have been linked to sex-related differences in autoimmunity, outcomes during acute and chronic infections, and responses to vaccination and immunotherapy.[294,295–303]

Sex hormones might contribute to these observed trends, but the story is complicated. In a cross-sectional study of people with and without long COVID stratified by sex assigned at birth according to healthcare records, females presented with distinct symptoms compared with males.[293] Compared with males, females had higher severity of symptoms, more neurologic disease, and more pain, among other differences. Males with long COVID had lower estrogen levels than males without long COVID, and females with long COVID had lower testosterone levels than females without long COVID. Across both cohorts, low testosterone was a strong predictor of immunologic dysfunction/inflammation and clinical disease.[293]

Sex differences and the role of sex hormones may provide clues to the biology of the condition. For example, interferon responses are known to differ between men and women[304–312]; these responses may be helpful in promoting viral clearance during acute infection but may also allow for the establishment of chronic infection.[313] Sex differences in type I interferon production in response to single-stranded RNA viruses can result in differences in disease manifestations.[314] Other factors, such as incomplete X chromosome activation and autoimmunity targeting Xist ribonucleoprotein complexes, are important in other autoimmune diseases.[294] Downstream pathways that that may be beneficial in acute infection may be also generate autoreactive immunity, as shown in lupus.[315]

Gender and sex balances in long COVID clinical trials will be important. Pre-specified hypotheses and analytic plans related to the role of sex should be a routine part of phase II and III studies, and sex should even be considered in smaller, probe studies. Indeed, a recent study of an RNAase (RSLV-132) showed a sex-related outcome in which individuals self-identifying as female disproportionately benefited from treatment.[316] Therapies that target sex hormones and other factors that can regulate the pathways of interest will need to be carefully considered.

The need for a surrogate biomarker

The lack of a viable biomarker for long COVID makes developing therapeutics challenging (Table 1). If viral persistence causes long COVID, then presumably a direct measure of the virus in blood or tissues (RNA, protein) will be discovered and validated, thus providing trialists with a reliable way to identify those who might have the disease (RNA- or antigen-positive) and perhaps even a way to quickly determine if an intervention is working (reduction in RNA or antigen levels). Other putative biomarkers being pursued include measures of inflammation, complement, and circulating microclots.[196] One study found changes in the brain's microstructure between people with long COVID and healthy controls using multi-compartment diffusion microstructural imaging (DMI); such efforts may also be helpful in the search for a long COVID biomarker. Given that there seems to be several long COVID endotypes, it is possible that there will prove to be several biomarkers or that combinations of markers may be needed.

The validation of any biomarker would greatly simplify drug development and make this space attractive to the biotechnology and pharmaceutical industry. Ideally, a biomarker would be a direct measure of the disease, predict who is at risk, and determine who is responding clinically to any intervention. Such a robust test would then be considered a surrogate marker for the disease, and regulatory approval for any new intervention might be achieved simply by proving this marker is effectively modified. Ultimately, unless clinical benefit is seen immediately with treatment, the identification of a surrogate marker would rapidly accelerate the development of therapeutics. This type of progression parallels the development of HIV therapeutics: prior to the validation of plasma HIV RNA as a surrogate marker, drug development required demonstrating that an intervention prevented progression to AIDS and death, rather than simply reducing HIV RNA levels in a durable manner. Once a surrogate marker for HIV was identified, industry engagement was massive, and dozens of effective drugs were rapidly tested and approved. A surrogate marker for long COVID would transform the field.

Identifying such markers is not easy—some parameters known to be associated with HIV progression (e.g., CD4+ T cell count) turned out to have great prognostic value but were not useful for guiding the development of therapeutics. Although efforts to identify such markers for other IACCs have come up short, the unique circumstances of long COVID may allow us to overcome many of the obstacles that were faced in other conditions. In addition, the availability of modern technologies, from ultrasensitive, high-parameter laboratory assays to advanced imaging techniques to machine learning, provides hope that it may yet be possible to identify a biomarker for long COVID. Success in this effort could also transform diagnostics for other IACCs.

Experimental medicine to probe the mechanisms

The communities of patients, researchers, and clinicians all recognize the urgency of getting therapeutic studies for long COVID into the clinic. We believe that there are sufficient data to support the testing of several potential mechanisms. We also believe that several FDA-approved drugs can be safely moved into proof-of-concept early-phase testing, both to be

Table 1. Biomarkers for long COVID

Type of biomarker	Description	Example
Mechanistic biomarker	identifies a root biological cause	a researcher identifies a consistent pattern in markers related to a specific biological pathway they are investigating as a cause of long COVID (e.g., spike protein, inflammation, and microclots)
Diagnostic biomarker	confirms that a person has a condition or confirms that a symptom is due to a specific condition	(1) a patient experiencing post-COVID-19 fatigue completes a panel of tests that confirms that they have a profile of measurements similar to other patients with long COVID; (2) a patient experiencing shortness of breath after COVID-19 undergoes a diagnostic test that confirms the symptom is specifically due to long COVID and not another condition (e.g., asthma)
Predictive biomarker	a measurement taken now predicts disease status in the future	(1) a measurement during the first 2 weeks of a SARS-CoV-2 infection predicts whether someone will have long COVID 3 months later; (2) a measurement in someone with long COVID now predicts whether they will spontaneously improve over the next year
Surrogate biomarker	altering the level in the short term can be anticipated to confer long-term benefit	by decreasing the level of a blood marker in the next month, a physician treating a patient with long COVID knows the patient will no longer have symptoms in a year

used as "probes" to explore the mechanism of long COVID and as part of early-stage studies to develop a product that might cure or reverse long COVID.

Interpreting the results of these studies may be challenging, given the lack of a biomarker strongly associated with long COVID and the lack of a consensus on how best to use patient-reported outcomes, essentially surveys exploring how participants feel and function. The likelihood that different endotypes of long COVID might be driven by different mechanisms warrants careful consideration of whether a study should define its eligible population broadly or narrowly. This might include the specificity of symptoms (e.g., any long COVID symptom versus a specific subtype of symptoms) and whether a participant should be required to meet certain objective criteria (e.g., self-reported symptom versus an objective deficit on physiologic testing). In addition, there are important considerations regarding the prior documentation of SARS-CoV-2 infection, which is a powerful concern for those who developed long COVID early in the pandemic before widespread testing was available. Excluding individuals without documentation of the initial infection could result in a study that many perceive to be exclusive. However, including such individuals may make a small, marginally powered study impossible to interpret, since a subset of those who believe they may have long COVID may not in fact have even been exposed to the virus. A final consideration is the necessity of a control arm. Although we have extensive experience with single-arm studies in other areas, we believe that the highly variable nature of long COVID requires a placebo group for the results to be interpretable. This is especially true in the absence

of an established biomarker; a number of recent studies demonstrated profound placebo effects.[109,224,316] Designs that incorporate delayed randomization, cross-over design, or a secondary open-label phase may therefore be favorable. Such designs provide the opportunity for all participants to eventually receive the intervention and might improve enrollment and retention, even if they add to complexity and cost. If single-arm studies are pursued, the rationale for this approach should be clearly justified and the findings confirmed in randomized trials.

We believe that the implementation of these small, intensive experimental medicine studies is essential as we move from the laboratory into the clinic. Such studies are unlikely to be powered to achieve a specific clinical outcome (although these can still be assessed) but instead rely on biological measurements such as changes in immune responses or other non-clinical tests. If such a study validates a targetable pathway as a potential cause of long COVID, then experience suggests industry will become highly engaged, and drugs specifically designed to reverse long COVID will move into early- and late-stage testing. Support for such studies has to date been limited, as large networks like RECOVER have leveraged their scale to conduct studies of hundreds of individuals. We encourage the development of funding mechanisms to support smaller-scale proof-of-concept studies that can inform what approaches should later be pursued in the networks.

Addressing regulatory issues

Issues related to regulatory considerations are part of the reason why long COVID trials have lagged behind those for acute

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Table 2. Treatments for COVID-19 and long COVID approved or authorized by the US Food and Drug Administration

COVID-19 prevention	COVID-19 treatment	Long COVID prevention and treatment
COVID-19 vaccine, mRNA (BioNTech); COVID-19 vaccine, mRNA (Moderna); COVID-19 vaccine, adjuvanted (Novavax); tixagevimab/cilgavimab[a]; pemivibart	nirmatrelvir/ritonavir; molnupiravir; remdesivir; casirivimab/imdevimab[a]; bamlanivimab[a]; bamlanivmab/etesevimab[a]; bebtelovimab[a]; sotrovimab[a]; baricitinib; tocilizumab; anakinra; vilobelimab	none

[a]Deauthorized as resistant SARS-CoV-2 variants emerged.

COVID-19. Regulatory agencies went to great lengths to facilitate the rapid implementation of acute COVID-19 trials, which benefited from an easy-to-define condition (COVID-19), easy-to-measure outcomes (hospitalization, death) that were observed over a short interval (days to weeks), highly engaged industry partners, and a friendly regulatory environment. Authorization and approval of multiple antiviral and immunomodulatory agents was achieved in a remarkably short time (Table 2).

There is much less certainty regarding long COVID, which (1) lacks a consistent case definition or validated biomarker, (2) is driven by a complex, as yet incompletely defined mechanism, (3) is measured clinically with patient-reported outcomes, which are poorly validated in this condition, (4) has a waxing and waning course that differs from person-to-person, leading to a need for large sample sizes, (5) might require weeks to months of treatment, and (6) has not received the same degree of regulatory and political support as was observed in 2020–2021 for COVID-19.

The FDA might consider options that would expedite the review of treatments intended to treat long COVID. This could involve use of fast track or breakthrough therapy designations or expanded access. Ultimately, a long COVID clinical trials agenda will require coordination between funders, regulators, investigators, and industry partners at the same level as was achieved for acute COVID-19.

Quantifying effectiveness and the need to define a target product profile

Until biomarker endpoints that can be used for regulatory approval can be identified, clarity on what patient-reported and/or quality-of-life endpoints might be acceptable would also be helpful. In the absence of a surrogate biomarker, serious consideration needs to be given to what would be required to deem a treatment for long COVID "effective." A statistically significant improvement in the report of symptomatology may or may not be clinically relevant. Recently, a study targeting the microbiome in those with long COVID noted greater alleviation of several symptoms including fatigue, issues with memory and concentration, and GI upset among those receiving the active intervention in comparison to placebo.[224] Yet the overall quality of life at the end of the study was similar between the treatment and control groups. Similar results have been found in clinical trials of treatments for related conditions like POTS, in which certain types of symptoms were improved but general health remained unchanged.[267] While small studies might only be powered to detect the potential for large changes, this might be a more effective approach than massive, expensive studies that detect small but less meaningful improvements. Given the extent

of the problem, however, both approaches may have merit. Guidance from regulatory agencies (as above) will be key, but because of these issues, efforts to develop a target product profile[317] for long COVID therapeutics outlining the desired attributes of potential treatments, including their safety and efficacy, are urgently needed.

COMBINATION STUDIES

It may take years to map the interplay of the multiple competing mechanisms of long COVID. Clinical trials are typically iterative, comparing single therapies to placebo in trials of increasing size to determine an effect, and then carefully combining treatments that suggest benefit in isolation to see if their use together may result in additive benefits. Based on the current understanding of long COVID, progression to combination trials may need to be accelerated: for example, intervening on multiple causes (e.g., SARS-CoV-2 persistence and clotting dysfunction, or targeting the virus and immune system simultaneously) as well as more downstream physiologic processes (e.g., endothelial dysfunction or the gut-brain axis). The pathway to such trials is long, and efforts to accelerate such approaches when they can be tested safely should be considered.

CONCLUSIONS

The SARS-CoV-2 pandemic has been framed as a once-in-a-century challenge. Long COVID is a challenge of similar scale. While long COVID is far from the first IACC affecting a large number of people, it is the first time that so many people developed such a condition simultaneously, following a known, shared, easily identified exposure. The scale of suffering is huge, and there is an urgent need for all types of research: epidemiology, basic, translational, and clinical science, and eventually implementation science, to get answers regarding the natural history, biology, and treatments for this condition. Long COVID is also unlikely to be the last of these challenges. Investment in efforts to understand this condition could benefit those who have suffered from similar conditions previously and has the potential to benefit millions after future pandemics.

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